

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 3, line 21 with the following:

a Recently, Arioli *et al.* (1998) cloned a *CelA* homolog, *RSW1* (radial swelling) (GenBank No. AF027172), from *Arabidopsis* by chromosome walking to a defective locus of a temperature sensitive cellulose-deficient mutant. Complementation of the ~~rsw1~~-*RSW1* mutant with a wild type full-length genomic *RSW1* clone restored the normal phenotype. This complementation provided the first genetic proof that a plant *CelA* gene encodes a catalytic subunit of cellulose synthase and functions in the biosynthesis of cellulose microfibrils. The full-length *Arabidopsis* *RSW1* represents the only known, currently available cellulose synthase cDNA available for further elucidating cellulose biosynthesis in transgenic systems (Wu *et al.*, 1998).

[Please replace the paragraph beginning at page 6, line 24 with the following:]

u2 An "isolated" nucleic acid molecule or polynucleotide refers to a component that is removed from its original environment (for example, its natural environment if it is naturally occurring). An isolated nucleic acid or polypeptide may ~~contains~~ contain less than about 50%, preferably less than about 75%, and most preferably less than about 90%, of the cellular components with which it was originally associated. A polynucleotide amplified using PCR so that it is sufficiently and easily distinguishable (on a gel, for example) from the rest of the cellular components is considered "isolated". The polynucleotides and polypeptides of the invention may be "substantially pure," *i.e.*, having the highest degree of purity that can be achieved using purification techniques known in the art.

[Please replace the paragraph beginning at page 7, lines 16-17 with the following:]

u3 A "function-conservative variant" is a polypeptide (or a polynucleotide encoding the polypeptide) having a given amino acid residue that has been changed without altering the overall conformation and function of the polypeptide, including, but not limited to, replacement of an amino acid with one having similar physico-chemical properties (such as, for example, acidic, basic, hydrophobic, and the like). Amino acids ~~with have~~ having similar physico-chemical properties are well known in the art. For example, arginine, histidine and lysine are hydrophilic-basic amino acids and may be interchangeable. Similarly, isoleucine, a hydrophobic amino acid, may be replaced with leucine, methionine or valine. Sequence-

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cont and function-conservative variants are discussed in greater detail below with respect to degeneracy of the genetic code.

94 [Please replace the paragraph beginning at page 10, line 14 with the following:]

The present invention relates to isolation and characterization of polynucleotides encoding cellulose synthases from plants, especially trees, including full length or naturally occurring forms of cellulose synthases, functional domains, promoters and regulatory elements. Therefore, in accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And Translation* [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; ~~*Immobilized Cells And Enzymes* [IRL Press, (1986)]~~; B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

95 [Please replace the paragraph beginning at page 11, line 2 with the following:]

The present invention relates to polynucleotides which comprise the nucleotide sequence that encodes cellulose synthase of the invention or a functional fragment thereof. In a ~~preferred~~ suitable embodiment, the polynucleotide comprises the sequence encoding a tree cellulose synthase and most ~~preferably~~ suitably, the sequence encoding a cellulose synthase from aspen. In one ~~embodiment~~ aspect, a polynucleotide of the invention includes the entire cellulose synthase coding region, e.g., nucleotides 69 to 3,005 of SEQ ID NO: 1. In another aspect of the invention, the polynucleotide encoding an *Arabidopsis* cellulose synthase is provided (see SEQ ID NO:4 and the translated protein of SEQ ID NO:5).

[Please replace the paragraph beginning at page 11, line 31 and bridging over to page 32 with the following:]

96 The polynucleotides that occur originally in nature may be isolated from the organisms that contain them using methods described herein or well known in the art. The non-naturally occurring polynucleotides may be prepared using various manipulations known in the field of recombinant DNA. For example, the cloned *CelA* polynucleotide can be

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modified according to methods described by Sambrook et al., 1989. The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the modified polynucleotides, for example, care should be taken to ensure that the modified polynucleotide remains within the appropriate translational reading frame (if to be expressed) or uninterrupted by translational stop signals. As a further example, a *CelA*-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Preferably, such mutations enhance the functional activity of the mutated *CelA* polynucleotide. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (~~Hutchinson, C., et al., 1978, J. Biol. Chem. 253:6554~~; Zoller and Smith, 1984, DNA 3:479-488; Oliphant et al., 1986, Gene 44:177; Hutchinson et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83:710), use of TAB linkers (Pharmacia), etc. PCR techniques are preferred for site directed mutagenesis (see Higuchi, 1989, "Using PCR to Engineer DNA", in *PCR Technology: Principles and Applications for DNA Amplification*, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70).

[Please replace the paragraph beginning at page 17, line 1 with the following:]

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The location of MSRE elements in the SEQ ID NO:3 may be identified, for example, using promoter deletion analysis, DNase Foot Print Analysis, and Southwestern screening of an expression library for an MSRE. In one embodiment, cellulose synthase promoter that has one or more portions deleted, and is operatively linked to a reporter sequence, is introduced into a plant or a plant cell. A positive MSRE is detected by observing no relative change or increase in the amount of reporter in a transgenic plant or tissue, *e.g.*, phloem after inducing a stress to the plant, and a negative MSRE is detected by observing increases in the amount of reporter in the plant in the absence of any stress to the plant. A positive element is detected when by removing it, GUS expression goes down and by adding it kept at the same level or more. The negative element does not support, or ~~suppresses~~ suppress, expression of GUS and by removing it, normal or enhanced GUS expression is observed as compared to when negative element is present.

[Please replace the paragraph beginning at page 20, line 27 with the following:]

28 The nucleotide sequence may be inserted in a sense or antisense direction depending on the needs of the practitioner. For example, if augmentation of cellulose biosynthesis is desired then polynucleotides encoding, *e.g.*, cellulose synthase, can be inserted into the expression vector in the sense direction to increase cellulose synthase production and thus cellulose biosynthesis. Alternatively, if it is desired that cellulose biosynthesis is reduced or lignin content is increased, then polynucleotides encoding, *e.g.*, cellulose synthase, can be inserted in the antisense direction so that upon transcription the antisense mRNA hybridizes to other complementary transcripts in the sense orientation to prevent translation. In other embodiments, the polynucleotide encodes a UDP-glucose binding domain and is used in a similar manner as described.

[Please replace the paragraph beginning at page 22, line 34 bridging over to page 23 with the following:]

29 Any non-tumorigenic *A. tumefaciens* strain harboring a disarmed Ti plasmid may be used in the method of the invention. Any *Agrobacterium* system may be used. For example, Ti plasmid/binary vector system or a cointegrative vector system with one Ti plasmid may be used. Also, any marker gene or polynucleotide conferring the ability to select transformed cells, callus, embryos or plants and any other gene, such as, for example, a gene conferring resistance to a disease, or one improving cellulose content, may also be used. Any promoter desired can be used, such as, for example, a *PtCelAP* of the invention, and those promoters as described above. A person of ordinary skill in the art can determine which markers and genes are used depending on particular needs.

[Please replace the paragraph beginning at page 26, line 6 with the following:]

30 The ~~full-length~~ full-length cDNA was designated *PtCelA*, and encodes a 110,278 Da polypeptide having an isoelectric point (pI) of 6.58 and 8 charged molecules. The hydropathy curve indicated that this particular cellulose synthase has eight transmembrane binding domains; two at the amino terminal and six at the carboxyl terminal, using the method of Hoffman and Stoffel (1993). This protein structure is analogous to those of RSW1 and GhCelA. All of the conserved domains for UDP-glucose binding, such as QVLRW and conserved D residues, are also present in a cellulose synthase of the invention, *e.g.*, *PtCelA* (Brown *et al.*, 1996). Thus, based on sequence and molecular analyses, it was concluded that

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PiCelA encodes a catalytic subunit which, like *RSW1* in *Arabidopsis*, is essential for the cellulose biosynthesis machinery in aspen.

[Please replace the paragraph beginning at page 27, line 25 with the following:]

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Xylogenesis in higher plants offers a unique model that involves sequential execution of cambium cell division, commitment to xylem cell differentiation, and culmination in xylem cell death (Fukuda, 1996). Although primary and secondary xylem cells originate from different types of cambia, namely procambium and inter/~~intrafascicular~~ intrafascicular cambium, both exhibit conspicuous secondary wall development with massive cellulose and lignin deposition (Easu, K., 1960, Anatomy of Seed Plants, New York: John Wiley and Sons Esau, 1965). To further investigate spatial and temporal cellulose synthase gene expression patterns at the cellular level, *in situ* hybridization was used to localize cellulose synthase mRNA along the developmental gradient defined by stem primary and secondary growth.

[Please replace the paragraph beginning at page 28, line 25 with the following:]

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During the primary growth stage (Fig. 3, panels a and b), strong expression of cellulose synthase was found localized exclusively to primary xylem (PX) cells. At this stage, young internodes are elongating, resulting in thickening of primary xylem cells through formation of secondary walls (Easu, K., 1960, Anatomy of Seed Plants, New York: John Wiley and Sons Esau, 1968). The concurrence of shoot elongation with high expression of cellulose synthase strongly suggests the association of cellulose synthase protein with secondary cell wall cellulose synthesis. Later stages of primary growth (Fig. 3, panel b) are characterized by the appearance of an orderly alignment of primary xylem cells. Active cellulose biosynthesis accompanies cell elongation-induced wall thickening, as indicated by the strong expression of cellulose synthase in these primary xylem cells.

[Please replace the paragraph beginning at page 30, line 11 with the following:]

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Characterization of promoter activity and cellular expression of a cellulose synthase of the invention from one particular source (aspen) indicated ~~hat~~ that expression produces a protein that encodes a secondary cell wall-specific cellulose synthase and is specifically compartmentalized in developing xylem cells. Characterization of the cellulose synthase gene promoter sequence not only confirms cell type-specific expression of cellulose

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synthase, but also provides a method for over-expressing cellulose synthase in a tissue-specific manner to augment cellulose production in xylem.

At page 34, line 19 please replace the citation in the section entitled Bibliography, with the following:

~~Esau, 1967, Plant Anatomy, Wiley and sons, NY~~

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Easu, K., 1960, Anatomy of Seed Plants, New York: John Wiley and Sons
